PCT/AUAU97/00367

Patent Office
Canberra

PRIORITY DOCUMENT

I, DAVID DANIEL CLARKE , ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed is a true copy of the Provisional specification as lodged on 11 June 1996 in connection with Application No. PO 0390 for a patent by NORTHERN SYDNEY AREA HEALTH SERVICES lodged on 11 June 1996.

I further certify that the annexed specification is not, as yet, open to public inspection.

WITNESS my hand this Nineteenth
day of June 1997

DAVID DANIEL CLARKE
ASSISTANT DIRECTOR PATENT SERVICES

AUSTRALIA

Patents Act 1990

NORTH SYDNEY AREA HEALTH SERVICE

PROVISIONAL SPECIFICATION

Invention Title:

Novel immunomodulatory t-cell antigen receptor clonotypic inter-chain disulphide peptides

The invention is described in the following statement:

Novel immunomodulatory t-cell antigen receptor clonotypic inter-chain disulphide peptides

The present invention relates to amino acid sequences designed to interfere with the function of the T-cell antigen receptor, such that these peptides can be used in the treatment of various inflammatory and autoimmune disease states. In particular , the peptides are useful in the treatment of disorders where T-cells are involved or recruited.

Background and introduction to invention

T-cells are a subgroup of cells which together with other immune cell types (polymorphonuclear, eosiniphils, basophils, mast cells, B-NK cells), constitute the cellular component of the immune system. Under physiological conditions T-cells function in immune surveillance and in the elimination of foreign antigen. However, under pathological conditions there is compelling evidence that T-cells play a major role in the causation an propagation of disease (listed in page 4). In these disorders, breakdown of T-cell immunological tolerance, either central or peripheral is a fundamental process in the causation of autoimmune disease. Central tolerance involves thymic deletion of self reactive cells (negative selection) and positive selection of T-cells with low affinity for self major histocompatibility complex antigens (MHC). In contrast, there are four, non-mutually exclusive hypotheses that have been proposed to explain peripheral T-cell tolerance which are involved in the prevention of tissue specific autoimmune disease. These include: anergy (loss of co-stimulatory signals, down regulation of receptors critical for T-cell activation), deletion of reactive T-cells, ignorance of the antigen by the immune system and suppression of autoreactive T-cells. Tolerance once induced does not necessarily persist indefinitely. A breakdown in any of these mechanisms may lead to auto-immune disease.

Autoimmune disease and other T-cell mediated disorders are characterised by the recruitment of T-cells to sites of inflammation. T-cells at these sites, couples with their ability to produce and regulate cytokines and influence B-cell function, orchestrate the immune response and shape the final clinical outcome. An understanding of the process of T-cell antigen recognition and subsequent T-cell activation, leading to T-cell proliferation and differentiation, is therefore pivotal to both health and disease. Disturbance in this intricate structure-function relationship of the T-cell antigen receptor, harmonising antigen recognition with T-cell activiation

may provide the therapeutic means to deal with inflammation and T-cell mediated disorders.

The critical component of antigen recognition on the surface of T-cells is the complex antigen receptor (TCR) which is a multisubunit structure that recognises antigen in the context of MHC-encoded proteins on the surface of antigen-presenting cells. The TCR is composed of at least seven transmembrane proteins. The disulfide-linked (αβ-Ti) hererodimer forms the clonotypic antigen recognition unit, while the invariant chains of CD3, consisting of ε, γ, δ, and ς and η chains, are responsible for coupling the ligand binding to signalling pathways that result in T-cell activation and the elaboration of the cellular immune responses. Despite the gene diversity of the TCR chains, two structural features are common to all known subunits. Firstly, they are transmembrane proteins with a single transmembrane spanning domain - presumably α-helical. Secondly, all the TCR chains have the unusual feature of possessing a charged amino acid within the predicted transmembrane domain. The invariant chains have a single negative charge conserved between the mouse and human and the variant chains possess one (TCR-β) or two (TCR-α) positive charges (Clevers et al., 1988).

Studies on the assembly of the multicomponent T-cell antigen receptor (Manolios et al., 1990, 1991, 1994) showed that the stable interaction between TCR-α and CD3-δ and TCR-α and CD3-ε was localised to eight amino acids within the transmembrane domain of TCR-α and it was the charged amino acids arginine and lysine that were critical. This finding exemplified the fact that amino acids within the transmembrane domain not only functioned to anchor proteins but were important in the assembly of subunit complexes and protein-protein interactions.

The above system depended on the modification of complementary strand DNA (cDNA) to create a number of protein mutants. Chimeric cDNA molecules were transfected into COS (fibroblast line) cells to express the required protein. Coexpression of these chimeric proteins were used to evaluate the region of interaction. Reiterating the above, the technology involved cDNA manipulation, metabolic labelling, immunoprecipitation and gel electrophoresis.

Transmembrane domains are small in size and proteins transversing this region are constrained to an alpha-helical configuration. These biophysical features pertaining to peptide and the region of interest coupled

with the ability to engineer protein-protein interactions via transmembrane charge groups suggested to the present inventor a possible new approach to intervene and potentially disturb TCR function.

The present inventor has now found that the extracellular site of protein-protein interaction between the two variant chains of the TCR is important in the function of the TCR. In particular, the present inventor has developed novel peptides which destabilise the interchain cysteine bond of the TCR-α and TCR-β chains and inhibit T-cell activation. The efficacious clinical manifestations of the administered peptide would be a decrease in inflammation e.g. decrease of arthritis in an adjuvant model of arthritis.

Accordingly, in a first aspect the present invention consists in a peptide which inhibits TCR function, wherein the peptide is of the following formula:-

R1-A-B-C-R2 in which

A is between 0 and 5 amino acids;

B is cysteine;

C is 4 or 5 amino acids;

R1 is NH2; and

R2 is COOH.

In a preferred embodiment of the present invention A is a peptide consisting of 5 amino acids.

In one embodiment the peptide is derived from the TCR-β chain. Preferably, C is 4 hydrophobic amino acids. In a preferred embodiment the peptide is of the following formula:-

NH2-Tyr-Gly-Arg-Ala-Asp-Cys-Ile-Thr-Ser-OH.

In another embodiment the peptide is derived from the TCR-α chain. Preferably, C is 5 hydrophobic amino acids. In a preferred embodiment the peptide is of the formula:

NH2-Ser-Ser-Asp-Val-Pro-Cys-Asp-Ala-Thr-Leu-Thr-OH.

In a second aspect the present invention consists in a therapeutic composition including a peptide of the first aspect of the present invention and a pharmaceutically acceptable carrier.

In a third aspect the present invention consists in a method of treating a subject suffering from a disorder in which T-cells are involved or recruited, the method including administering to the subject a

therapeutically effective amount of the peptide of the first aspect of the present invention.

The therapeutic composition may be administered by any appropriate route as will be recognised by those skilled in the art. Such routes include oral, transdermal, intranasal, parenteral, intraarticular and intaocular.

As used herein, the term "subject" is intended to cover both human and non-human animals.

As will be readily understood by those skilled in this field hydrophobic amino acids are Ala, Val, Leu, Ile, Pro, Phe, Tyr and Met.

A non-exhaustive list of disorders in which T cells are involved/recruited include:

- Allergic diathesis e.g. Delayed type hypersensitivity, contact dermatitis
- Autoimmune disease e.g. SLE, rheumatoid arthritis, multiple sclerosis, diabetes, Guillain-Barre syndrome, Hashimotos disease, pernicious anaemia
- Gastroenterological conditions e.g. Inflammatory bowel disease, Chrons disease, primary biliary cirrhosis, chronic active hepatitis
- Skin problems e.g. psoriasis, pemphigus vulgaris
- Infective disease e.g. AIDS virus, herpes simplex/zoster
- Respiratory conditions e.g. allergic alveolitis,
- Cardiovascular problems e.g. autoimmune pericarditis
- Organ transplantation
- Inflammatory conditions e.g. myositis, ankylosing spondylitis
- Any disorder where T cells are involved/recruited.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

DATED this 11th day of June 1996

NORTHERN SYDNEY AREA
HEALTH SERVICE
Patent Attorneys for the Applicant:

F.B. RICE & CO.

References

Clevers H., Alarcon B., Wileman T., Terhorst C (1988). Am Rev. Immunol. 6,629

Cosson P., Lankford S.P., Bonifacino J.S., Klausner R., (1991). Nature 351, 414

Manolios N., Bonifacino J.S., Klausner R.D., (1990) Science, 248, 274

Manolios N., Letourner F., Bonifacino J.S., Klausner R.D. (1991) EMBO J.10 1643